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THE PRODUCTION OF TETRAPLOID REDWING FLAX AND TETRAPLOID F_1 HYBRIDS OF A NUMBER OF CROSSES INVOLVING THIS VARIETY

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THE PRODUCTION OF TETRAPLOID REDWING FLAX AND TETRAPLOID F_1 HYBRIDS OF A NUMBER OF CROSSES INVOLVING THIS VARIETY

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INTRODUCTION

Cultivated flax, Linum usitatissimum L., is used as a source of farm revenue to only a limited extent in northern Alberta despite the attractive price and immediate need of oil derived from flaxseed. The variety Redwing is suitable from the standpoint of earliness, strength and length of straw, and other general considerations, but has very small seed. This last, besides causing unpopularity with the farmer, contributes to difficulties in storage, cleaning, and transportation. Small-seeded varieties inevitably have low yields of oil, making this variety unpopular with the processor in spite of the high quality of the oil obtained.

Two possibilities for increasing the size of Redwing seed are available. Repeated backcrossing of large-seeded, hybrid selections to Redwing, after the initial cross with a large-seeded, desirable variety, offers the opportunity of obtaining a large-seeded Redwing type at the end of a number of years of breeding and selection. The other possibility is to obtain a strain of tetraploid Redwing. In order to maintain

fertility in the autotetraploid heterozygosity before doubling may be necessary. Seed size has been shown to increase appreciably when the chromosome number of a species is doubled. The first method was started but, owing to the long-term nature of the program, it could not be completed. This study consists of an investigation of the second method.

By the use of colchicine tetraploids of Redwing and of F_1 plants of nine different crosses involving Redwing were obtained. Chromosome numbers of the diploid parents and of the tetraploid Redwing and tetraploid hybrids were determined. Observations were made on growth habits and fertility of the tetraploid in comparison with the diploid forms.

LITERATURE REVIEW

The chromosome number of cultivated flax, Linum usitatissimum L., has been found to vary in different varieties. An early paper by Tammes (21) cited the work of Reinder who found that L. usitatissimum L. and L. angustifolium Huds. each had 30 somatic chromosomes. This same number was obtained by Vilmorin and Simonet (22) in an examination of meiotic figures in which n = 15. Kikuchi (8) verified these findings with counts at the somatic and meiotic division. However, Martzenitzina (11) and Emme and Schepeljeva (5) reported 32 chromosomes in varieties of L. usitatissimum L. Emme and Schepeljeva (5) found both n = 15, 2n = 30, and n = 16, 2n = 32 in two different strains of L. usitatissimum L. var. crepitans. Muntzing and Runquist (13) report 2n = 31 and 2n = 32 in the same variety. Dillman (4) cites the determinations of Dr. A. E. Longley on two varieties, Hoshangabad C.I.40 (India) and Bison (North Dakota Agr. Exp. Sta.) of L. usitatissimum L. in which the haploid number was 15. In view of the inconsistency of the above findings it seems possible that L. usitatissimum contains races differing in chromosome numbers.

Tetraploid forms of L. usitatissimum have been reported by a number of workers, Simonet, Chopinet, and Souilijaert (19), Muntzing and Runquist (13), Gyorffy as reported by Muntzing and Runquist (13), Lutkov (10), and Ray (16).

Heat treatment of the zygote was successfully used by Lutkov in 1938 to produce a tetraploid flax plant, but in 1939 he found colchicine treatment much more effective (10).

The action of colchicine is on the dividing cell. The primary effect is the inhibition of spindle formation so that the chromosomes remain in the metaphase condition for a longer period of time than normally. Furthermore, complete division of the chromosome is inhibited by the failure of the centromere to divide when the rest of the chromosome divides--another factor which prevents anaphasic movement of the chromosomes. After sufficient recovery from the effects of colchicine, division is completed and the divided chromosomes reorganize into a single nucleus. Succeeding cell generations then contain the doubled chromosome complement (6).

Various methods of applying colchicine to the growing tip of plants in order to produce tetraploid tissue have been devised. Blakeslee and Avery (1) cite six methods that may be used. These are, briefly, the immersion of the growing tip in a solution of colchicine; brushing a 0.8% agar solution of .1% colchicine on the growing tip; the same treatment with lanolin solution; application by means of a capillary string; application of a single drop of colchicine solution at intervals on the growing tip; and atomizing the tip with colchicine solution. Germinating of seeds in dilute colchicine solution may in some cases give satisfactory results.

Lutkov (10) treated the stem side of germinating

seeds of flax, *L. usitatissimum*, but covered the roots with moist filter paper, not allowing them to come in contact with the 0.2% aqueous solution of colchicine. The mortality of seedlings was increased with the duration of the treatment, especially when the treatment extended to 48 hours. The total number of polyploids from the treatments of short duration (6, 12, 18, 24, and 36 hours) was higher than from those of long duration (48 hours). The percentage of tetraploid plants among those surviving varied from 42.8% to 83.3%. Metaphase plates from these tetraploids with 4n = 64 chromosomes were observed. The growing tips of other plants were treated with uncongealed drops of colchicine solution (0.05, 0.1, and 0.2%) in 0.6% agar. The results with this treatment were similar to those previously described.

Dona' Dalle Rose (3) treated germinating flax seed with solutions of 0.25%, 0.50%, and 1.0% colchicine for periods of 12, 18, 24, and 48 hours. Out of the surviving plants a number showed distinct mutant characters, but no attempt to determine their chromosome number was made.

Muntzing and Runquist (13) treated two different flaxseed portions with 0.025% colchicine solution--one for two and the other for four days. Four tetraploid plants with chromosome numbers of 58±, 59±, 60±, and 61± were obtained from the two-day treatment, while the two tetraploids obtained from the second treatment had 59± and 60± chromosomes. Simonet, Chopinet, and Souilijaert (19) treated an oil flax variety by soaking the seeds in 0.1%, 0.2%, and 0.4%

colchicine for 2, 4, and 6 days and found that the treated seeds developed slowly and only a small number survived. The largest number of tetraploids was obtained from the seed treated for two days with the 0.1% solution. An examination of meiosis at first division metaphase of a tetraploid was made and n = 30 observed. Simonet and Chopinet (18) brushed warm 3% agar with an equal quantity of 2% solution of colchicine on the epicotyl as it emerged from between the cotyledons of the L. grandiflorum seedlings. Two applications were made at four or five-day intervals and five percent of the seedlings were found to be tetraploid. These workers do not appear to be enthusiastic over seed treatment, saying that thousands of seeds so treated gave negative results.

Blakeslee and Avery (1) mention that the increased size of the pollen grains and guard cells is a fairly reliable indication of doubling as these are less affected by environment than other cells of the plant. Breslavez (2) states that no one irregular or enlarged plant part is an infallible indicator but a number of these observations are necessary before a tetraploid condition can be reliably assumed. Certain morphological characteristics, such as increased size of flowers, pollen, bolls, and seeds, were found by Lutkov (10) to be associated with the tetraploid condition in flax. Pollen size was found to be particularly useful in distinguishing tetraploid plants. The diameter of pollen grains in diploid forms of fiber flax, variety Pobeditel, varied from 45.9u to 68.8u, while that of pollen

grains of flowers on altered shoots reached 84.0-91.8u, indicating that these shoots were tetraploid. He further states that cytological analysis of the progeny of those plants which set seeds has shown that there is almost complete agreement between the data presumed on the basis of a study of pollen size and the data obtained by a study of the chromosome number. Simonet and Chopinet (18) found the volume of the pollen grains of the tetraploid to approximate twice the volume of the diploid grains.

Simonet, Chopinet, and Souilijaert (19) examined pollen mother cells of tetraploid flax, observing univalents, bivalents, trivalents, and tetravalents at heterotypic metaphase. The chromosomes were unequally apportioned at homotypic division and normal tetrads were sometimes replaced by the five- or six-celled condition. This explained the irregularities found in pollen size. In examining meiosis of a tetraploid oil flax, Ray (16) found a high frequency of multivalents which led to irregularities in the subsequent division and consequent reduction of fertility to one or two seeds per boll.

Lutkov (10) states that decreased numbers of seeds per capsule were found in the tetraploid plants. Newcomer (14) found that a colchicine induced tetraploid of Cosmos set no seed in the greenhouse but, under field conditions, was fully fertile. Muntzing (12) mentions the part that autopolyploidy may have played in evolution, pointing out the

opportunity for a fertile autopolyploid to be naturally selected from the many autopolyploids which may be produced from diploid species through natural agencies such as cold or heat. The possibility of selection for fertile types was illustrated by Kostoff (9). From an allopolyploid hybrid Nicotiana glauca x Nicotiana Langsdorffii of 50% fertility, in five generations a normally fertile strain was selected. After continually selecting plants with highest fertility, amphidiploids were produced in which the fertility was increased from 48 seeds per capsule in the first generation to 200 seeds per capsule in the fifth generation.

Randolph (15) doubled both homozygous and heterozygous strains of maize and found that in contrast to the pronounced deleterious effects of doubling homozygous inbred lines, the doubling of heterozygous open pollinated varieties or hybrids derived from inbred lines produced vigorous robust and highly fertile tetraploids. Working with autotetraploids of fifteen varieties of Antirrhinum and also with crosses of these autotetraploid varieties, Sparrow, Ruttle, and Nebel (20) found a marked increase in fertility of the latter in comparison with the former. Through observations at meiosis and examination of pollen grain fertility of the autotetraploids and allotetraploids, it was found that the sterility difference between the two types of tetraploids could not be attributed merely to meiotic irregularities or to pollen deficiencies.

MATERIALS

Since the purpose underlying this study was to obtain a large-seeded Redwing type flax, the simple expedient of doubling the chromosome number of this variety was attempted. Because of the possibility of infertility in the autotetraploid, however, it was thought advisable to double certain F_1 hybrids in order to increase heterozygosity and thereby probably obtain increased fertility. With this in view, a number of varieties were crossed with Redwing in the summer of 1941 and F_1 seedlings treated with colchicine in the fall and winter of 1941-42.

The varieties used were obtained from a number of sources. Redwing (C.A.N. 2239) was obtained from the stock of elite seed produced at the University of Alberta. This variety is early, medium tall, blue-flowered, and produces small brown seeds. Bison (C.A.N. 2100) and Crown (C.A.N. 2109) were originally obtained from the Cereal Division, Ottawa, in 1937. Royal, a selection from Crown made at the University of Saskatchewan was introduced in 1937. The last three varieties are much the same in growth habits, being medium early, medium tall, blue-flowered, and producing medium large brown seeds. Pale Blue, an early, medium tall, pale blue-flowered variety, also producing small brown seeds, was imported in 1933. Hoshangabad (C.I.40) and Indian Type 35 were introduced to this station in 1931. Hoshangabad is an early short

variety with pink flowers and large, yellow seeds. Indian Type 35 is also short and early with blue flowers and large brown seeds. L-33-1, L-33-14, and L-35-23 were obtained from the Lacombe Experimental Station in 1939. Both L-33-1 and L-33-14 were selected at Lacombe in 1933 from a variety named Walsh. In 1935, L-35-23 was selected at Lacombe from the F_2 or F_3 generation of a Long Sib. x Early Walsh cross made some time previously by the Cereal Division, Ottawa. These varieties are all medium tall, medium early, blue-flowered, and produce very large, brown seeds.

Quality determinations on these varieties grown at Edmonton were made by Dr. Sallans at the Oil Seeds Laboratory at Saskatoon (Table I). As indicated by the iodine value, Redwing, Pale Blue, Crown, and Hoshangabad produced oil of high quality. Hoshangabad is outstanding in that its oil content is the highest of all varieties--10% more than that of Redwing and Pale Blue. Along with these desirable qualities it has high 1000-kernel weight. L-33-1 and L-35-23, though having oil only fair in quality, have good oil content and the highest 1000-kernel weight. Pale Blue and Redwing have the lowest 1000-kernel weight.

TABLE I
Test of parents, 1941

Variety	N.S.N.	Oil content	Iodine number	1000-K weight
Redwing	I-37-13	38.8	191.0	4.53
Royal	I-37-22	41.0	184.5	5.40
Bison	I-37-4	40.9	185.7	5.71
Pale Blue	I-33-7	38.7	194.6	4.07
Crown	I-37-16	40.4	189.9	4.75
L-33-1		42.6	183.3	10.20
L-33-14		42.8	185.4	8.82
L-35-23		42.6	183.1	10.10
Indian Type 35	I-31-84	45.5	177.3	7.69
Hoshangabad	I-31-123	48.7	190.6	8.70

METHODS

To obtain tetraploid plants, application of very dilute colchicine solution was made on rapidly growing plant parts. A number of different methods of application were tested for their efficacy in producing tetraploids from diploid flax. The germinating seed treatment method was selected first because of its simplicity. In a Petri dish, 100 seeds were placed on a double layer of filter paper and 5 cc. of 0.2% or 0.4% aqueous solution of colchicine were measured into it. The Petri dish was placed in darkness at constant temperature and left for the desired number of hours. The seeds at the end of the treatment time were washed with distilled water and transferred to a clean filter paper in a clean Petri dish containing 5 cc. of distilled water. The Petri dish was left under the same conditions as before and after one or two days the seeds were planted.

Using this method the mortality was so great that a less destructive treatment was sought. One percent colchicine in 1.5% agar solution, as described by Simonet and Chopinet (18), was obtained by preparing separately 2% aqueous colchicine and 3% agar solution and, mixing these in equal proportions. While still warm, this solution was applied to the growing epicotyl as it became visible between the two cotyledons. This process was repeated with caution at intervals of 12 to 24 hours or more. Caution was observed

since an unduly prolonged treatment might kill the shoot.

The rapid drying and cracking of the agar on the epicotyl prompted the use of some other medium. Blakeslee and Avery (1) suggested the use of lanolin. A colchicine emulsion of lanolin was, therefore, prepared as follows:

Stearic acid - 1.50 gm.
Morpholine - 0.53 cc.
Tap water - 20.00 cc.
Lanolin - 8.00 gm.
Colchicine - 0.12 gm.

The water was added to the stearic acid and morpholine and heated until the stearic acid had melted. The mixture was then stirred to a creamy soap solution. The lanolin was added and heating continued until the mixture reached a temperature just below boiling point. The colchicine was then added and the mixture stirred until a thick, creamy emulsion resulted. The emulsion was stirred intermittently until it had reached room temperature.

For treatment of flax seedlings it was found advisable to dilute this emulsion to the desired consistency with 0.4% or 0.2% aqueous colchicine solution. The emulsion was applied with a brush to the epicotyl as it appeared between the cotyledons. Treatment was repeated at one- to three-day intervals. Care was taken to ensure that the shoot should not be killed through prolonged treatment.

The tetraploid shoots were recognized by microscopic examination of pollen grains mounted in permanent acetocarmine. An ocular micrometer was used to measure the size of

the pollen grains from flowers of each treated shoot.

The seeds from tetraploid shoots determined as above were sown in small pots. After the plant was growing vigorously root tips were taken. These tips were very small, however, and it was found that a larger, more satisfactory root tip could be procured by germinating the seed on filter paper in Petri dishes and taking the tip of the primary root after it was one-half to three-quarters of an inch long. The seedlings were then planted in moist soil, and despite this drastic treatment mortality was almost nil.

Karpechenko's solution was used to kill and fix the root tips and the paraffin method followed in preparing them for sectioning at 10u thickness.

In addition to this method a short method as described by Kerns (7) proved very satisfactory. This method made possible examination of the chromosomes on the same day that root tips were taken.

With the use of a crystal violet staining technique as described by Young (23) the chromosomes were stained so that they could be counted quite easily. His method is as follows:

"Bleach overnight in 3% H_2O_2 and 70% alcohol (1:1).
Wash two changes of 5 minutes each.
Stain 5 minutes in a 1% aqueous solution crystal violet.
Rinse in water.

Mordant 30 sec. in iodide 1 gm. iodine
1 gm. potassium iodide
100 cc. 80% alcohol.

Destain 8 sec. in 95% alcohol
10 sec. in 100% alcohol
3 to 8 minutes in clove oil.
Xylene three changes."

Another crystal violet staining technique as described by Sass (17) calling for a rinse in saturated picric acid solution in 100% ethyl alcohol before destaining with clove oil was also used. The picric acid set the stain in the chromosomes giving them a darker and more distinct appearance.

Counts were made of chromosomes in the metaphase plates in cells of the root tip. A number of such plates were examined to determine the true tetraploid character. Two of the best plates were selected and the chromosomes drawn on a card in their proper relation. If the counts made corresponded this number was taken as the true count. If not, sufficient plates were counted to be reasonably sure of the number. In some cases where no definite count could be established the numbers were marked ±.

Pollen mother cells of both diploid and tetraploid Redwing as well as of each parent variety of the hybrids were examined using permanent aceto-carmine stain as described by Zirkel (24):

Acetoocarmin	80 cc.
Karo corn syrup (dextrose)	10 cc.
Certo (pectin)	10 cc.

An anther of a flower bud approximately 1 mm. long was removed and placed in a drop of stain on a slide. The pollen mother cells were teased out with a flat-pointed steel needle. The slide was examined under low power and, if evidence of metaphase plates of the first division was detected, the preparation was left for at least five minutes. At the end of this time a cover glass was placed carefully on the drop of permanent aceto-carmine stain. The slide was placed on a hot water bath for approximately 45 seconds. This served to fix the stain in the chromosomes and clear the cytoplasm. Immediately after, while still hot, the slide was placed between blotting papers and sufficient pressure applied to flatten the material under the coverslip. The preparation could be examined immediately with the oil immersion lens if care were exercised to prevent the lens from pressing against the coverslip.

Certain morphological characteristics of the plants, such as flower diameter, boll diameter, petal size, and height, were measured. Stomatal size was obtained by carefully stripping the lower epidermis of leaves obtained from approximately the same place on each plant. Plants compared were at the same stage of growth. These strippings were mounted under a coverslip

in permanent aceto-carmine and measured immediately with an ocular micrometer. The stomata varied in size, being large toward the tip of the leaf and smaller toward the base, and this made it necessary to take measurements at approximately the same location on each leaf.

RESULTS

The Production of Tetraploid Redwing and F_1 Hybrids

Tetraploids of the Redwing variety were first obtained. Treatments of germinating seed as indicated in Table II caused very high mortality among the 8,900 germinating seeds. Of these treatments the 0.2% colchicine treatment for 24 hours in all cases gave the best results both in percentage survival and in percentage tetraploids among those which survived. The seven tetraploids obtained resulted from this particular treatment. This type of treatment caused a pronounced deformation of the seedling, particularly of the primary root, as illustrated in Figure 1.

A preliminary growing tip treatment as used by Simonet, Chopinet, and Souilijaert (19) caused no mortality among the seedlings treated. Of the 28 plants, three were tetraploid (10.7%) which is higher than the percentage of tetraploids surviving from any of the germinating seed treatments.

TABLE II

Colchicine treatment of germinating seed and growing tips of Redwing flax (1941)

Date treatment started	No. of seeds treated	% colchicine	Length of treatment (hours)	Temperature ($^{\circ}$C.)	Location	Plants surviving			
						Number	Percentage	No. of tetraploids	% of tetraploids
		Germinating seed treatment (colchicine in aqueous solution)							
April 4	200	0.2	24	16-17	Greenhouse	69	34.5	5	7.2
	200	0.2	48	16-17	"	1	0.5	0	0
	200	0.4	24	16-17	"	0	0	0	0
	200	0.4	48	16-17	"	0	0	0	0
May 13	200	0.2	24	15-16	"	37	18.5	2	5.4
	200	0.2	36	15-16	"	0	0	0	0
	200	0.4	18	15-16	"	7	3.5	0	0
May 22	2000	0.2	26	16-17	Field	2	0.1	0	0
	2000	0.2	37	16-17	"	1	0.05	0	0
	2000	0.4	23	16-17	"	0	0	0	0
June 11	500	0.2	24	16-17	Greenhouse	2	0.4	0	0
	500	0.2	36	16-17	"	1	0.2	0	0
	500	0.4	18	16-17	"	0	0	0	0
		Growing tip treatment (colchicine in 1.5% agar solution)							
June 14	28	1.0	*	21-30	Greenhouse	28	100.0	3	10.7

* Solution brushed twice on epicotyl of seedling, with 12-hour interval

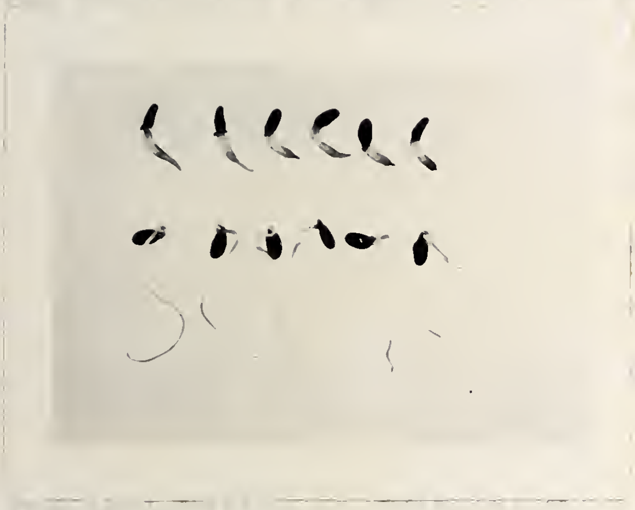

Figure 1

Effects of colchicine treatment on
germinating Redwing seed

Treated germinating seed (above) and control (below) are shown six days after germination began. Treatment consisted of soaking the seed 24 hours in 0.2% colchicine.

As indicated in Table III parent varieties and F_1 hybrid seedlings were treated with colchicine. Different lots of seeds were planted at different times and treated differently as the explanatory notes on the table indicate.

The colchicine in agar solution gave percentages of tetraploid shoots among those surviving, as follows: Treatment I, 5.1%; Treatment II, 11.5%; Treatment III, 4.1%; Treatment IV, 3.5%. Normally the number surviving under such treatment is high but, owing to lack of sunlight in the greenhouse during the winter, the plants were weakened and a large number--the majority in some cases-- were killed by damping off disease.

The application of colchicine in lanolin emulsion was the most efficacious in the production of tetraploid shoots. The best results were obtained from Treatments V and VII, both of which were lanolin treatments and from which 18.1 percent of tetraploid shoots was secured. A stunting and thickening of the stem and leaves of treated seedlings resulted from this colchicine-in-lanolin treatment as shown in Figure 2. A variety of malformations often occurred, of which Figure 3 is an example.

The germinating seed treatment (Treatment VI) again caused very heavy mortality and gave a small percentage of tetraploid plants.

In some cases, owing to conditions of temperature, sunlight, and humidity, very few bolls set on either the diploid or the tetraploid shoots. No viable seed was produced from

TABLE III

Colchicine treatment of growing tips and
germinating seeds of F_1 hybrids and
parent varieties
1941 - 1942

		Treatment						
		I	II	III	IV	V	VI	VII
Date planted		16/9	17/9	3/10	19/10	20/1	5/2	5/2
Crown	p*	11	10	10	10	8	31	14
x	s	4	3	9	10	8	3	11
Redwing	t	1	0	0	0	1	0	1
Bison	p	11	10	10	10	8	48	36
x	s	2	2	10	8	5	12	27
Redwing	t	0	1	0	0	0	0	6
Royal	p	11	10	10	10	8	50	50
x	s	3	4	10	10	6	10	30
Redwing	t	0	0	2	1	1	2	4
Pale Blue	p	11	10	10	10	8	29	25
x	s	2	1	9	8	7	14	18
Redwing	t	1	0	0	1	0	0	0
L-33-1	p	11	10	10	10	8	--	--
x	s	5	4	9	8	7	--	--
Redwing	t	0	1	0	0	2	--	--
L-33-14	p	11	10	10	10	8	30	25
x	s	3	1	10	9	6	3	17
Redwing	t	0	0	0	1	3	0	4
L-35-23	p	11	10	10	10	8	--	--
x	s	2	3	9	7	3	--	--
Redwing	t	0	1	1	0	0	--	--
Ind.Type	p	11	10	10	10	8	25	14
35 x	s	4	0	10	10	2	4	12
Redwing	t	0	0	1	0	1	0	8
Hoshan-	p	11	10	10	10	8	15	--
gabad x	s	1	1	10	9	5	0	--
Redwing	t	0	0	0	0	1	0	--

* p = number of seeds planted
s = number of plants surviving at maturity
t = number of tetraploid plants

TABLE III (Continued)

		Treatment						
		I	II	III	IV	V	VI	VII
Date planted		16/9	17/9	3/10	19/10	20/1	5/2	5/2
Redwing	p	5	5	5	5	4	76	3
	s	1	1	5	3	3	1	2
	t	0	0	0	0	1	0	0
Crown	p	6	5	5	5	4	72	3
	s	2	0	5	2	4	3	1
	t	0	0	0	0	0	0	0
Bison	p	2	5	5	5	4	76	-
	s	0	2	2	3	4	0	-
	t	0	0	0	0	0	0	-
Royal	p	3	5	5	3	4	72	2
	s	1	1	0	4	2	0	1
	t	0	0	0	0	0	0	0
Pale Blue	p	3	5	5	5	4	72	3
	s	1	0	5	5	1	0	3
	t	0	0	0	0	1	0	0
L-33-1	p	6	5	5	5	4	102	-
	s	2	0	5	0	4	1	-
	t	0	0	1	0	1	0	-
L-33-14	p	5	5	5	5	4	89	3
	s	3	1	5	5	2	0	3
	t	0	0	0	0	0	0	0
L-35-23	p	6	5	5	5	4	76	-
	s	2	2	2	5	0	0	-
	t	0	0	0	1	0	0	-
Indian Type 35	p	3	5	5	5	4	72	2
	s	0	0	5	5	2	0	2
	t	0	0	0	0	0	0	0
Hoshangabad	p	5	5	5	5	4	89	-
	s	1	0	3	4	1	1	-
	t	0	0	0	0	1	0	-
Total	p	144	140	140	140	112	1024	165
	s	39	26	123	115	72	52	127
	% s	27	19	88	82	64	5	77

TABLE III (Continued)

	Treatment						
	I	II	III	IV	V	VI	VII
Date planted	16/9	II 17/9	3/10	19/10	20/1	5/2	5/2
No. of tetraploids	2	3	5	4	13	2	23
% tetraploids of those surviving	5.1	11.5	4.1	3.5	18.1	3.8	18.1

Treatment I

1% colchicine in 1.5% agar solution was brushed on the epicotyl one day after emergence. This treatment was repeated three times at 24-hour intervals.

Treatment II

1% colchicine in 1.5% agar solution was brushed on the epicotyl one day after emergence and the treatment repeated four times at 24-hour intervals. At the end of the next 24-hour period, 1.5% colchicine was used. At the end of the next, the strength of the colchicine was raised to 2%.

Treatment III

1% colchicine in 1.5% agar solution was brushed on the epicotyl one day after emergence. The same strength was used 24 and 48 hours later. The strength of the colchicine was increased to 1.14% in a treatment 72 hours after the first and to 1.33% in a further treatment at the end of 96 hours.

Treatment IV

1% colchicine in 1.5% agar solution was brushed on the epicotyl one day after emergence. The same strength was applied at the end of periods of 22, 23, 93, and 144 hours from the first treatment.

Treatment V

0.4% colchicine in lanolin emulsion was brushed on the epicotyl one day after the seedlings emerged and, thereafter, at the end of 18, 48, 73, and 91 hours.

Treatment VI

Seeds were germinated in 0.2% aqueous solution of colchicine for 20 hours.

Treatment VII

The lanolin emulsion was thinned with 0.4% aqueous colchicine solution. This was applied to the epicotyl one day after emergence and again at the end of 24, 48, and 73-hour periods.

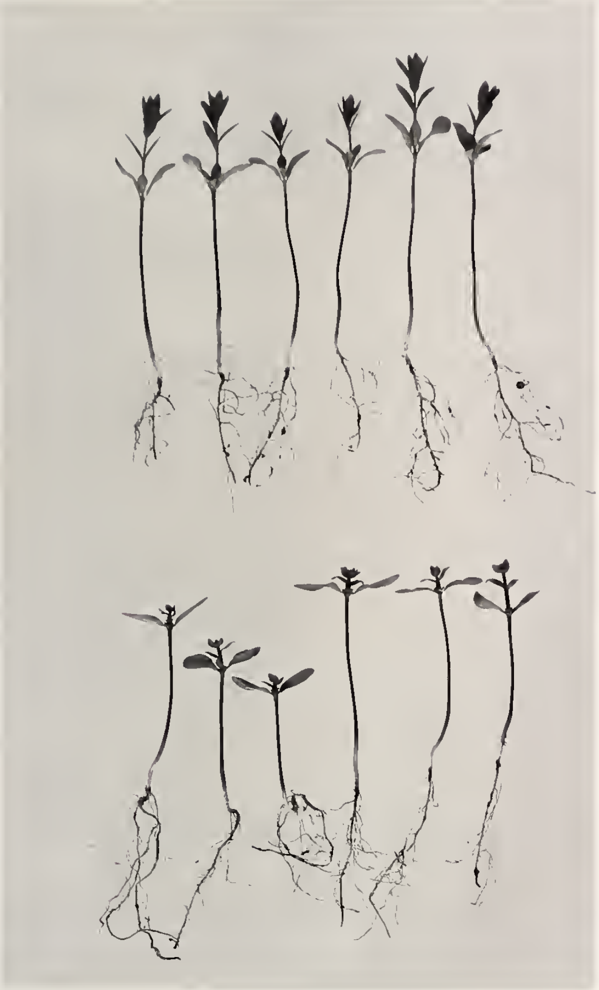

Figure 2

Effect of growing-tip treatment on
Redwing seedlings

Treated seedlings (below) and control (above) are shown seven days after the last treatment. Lanolin emulsion of 0.2% colchicine was applied to the epicotyl as it appeared between the cotyledons and again at the end of 24, 48, and 120 hours.

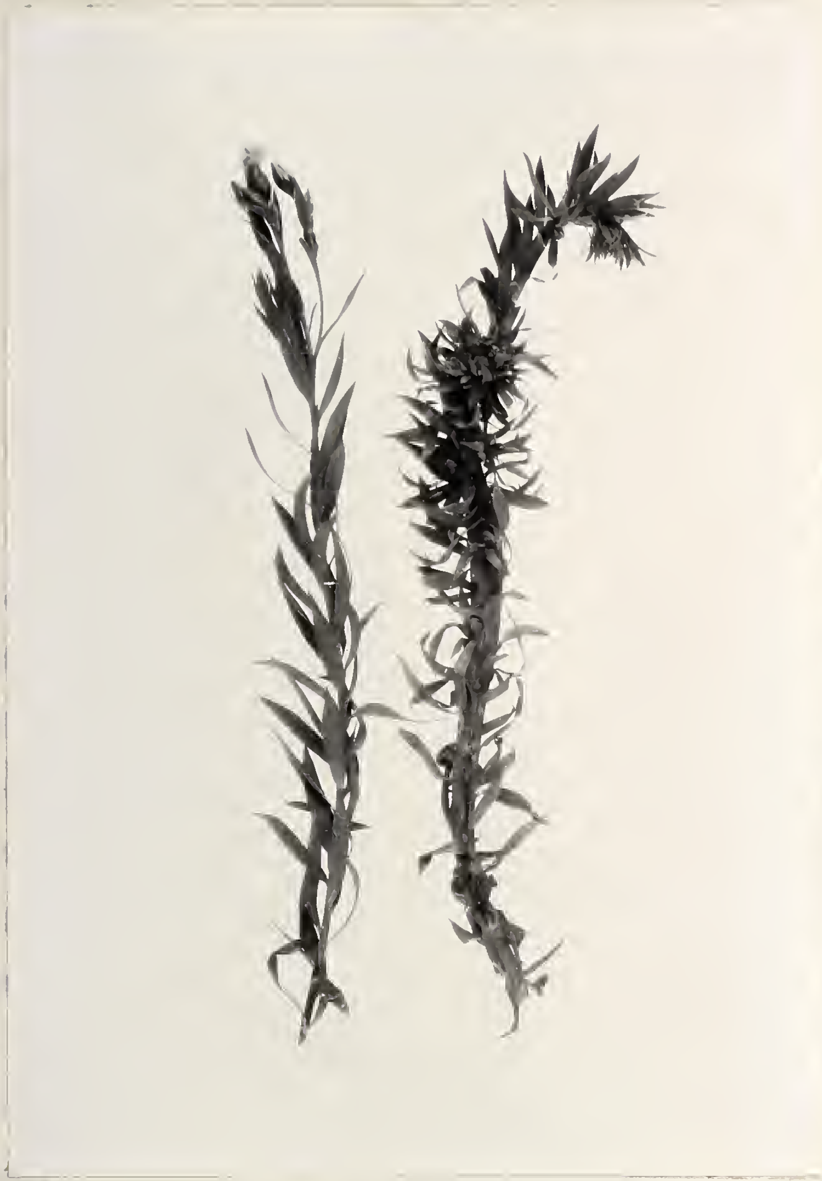

Figure 3

Normal shoot (left) and fasciated shoot (right), the latter being an example of one type of malformation found among the colchicine treated plants

tetraploid shoots of L-33-1, L-35-23, Hoshangabad, or Hoshangabad x Redwing F_1 hybrid.

Contrasting Morphological and Physiological Characters of Tetraploid and Diploid Plants of Redwing flax

On August 4, 1941, tetraploid and diploid seeds from treated shoots as well as a number of seeds from the original diploid stock were sown singly in 5-inch pots in the greenhouse. Chromosome numbers were determined on the majority of these and the stomata and pollen size used to classify the remainder as either tetraploid or diploid. Table IV gives the chromosome numbers as well as certain measurements and observations made on each plant. The chromosome numbers of the tetraploid plants in the majority of cases are 60, but in some cases differ by 1, 2, or 3 chromosomes more or less. Days to maturity were not recorded because of the prolonged vegetative period in the greenhouse.

In general appearance the tetraploid plants did not differ greatly from the diploid forms except that the leaves of the former tended to be thicker, giving the plants a darker green shade than that of the diploid.

The tetraploid plants were, on the average, 17.7 days or 12.2% longer in coming into first flower than the diploid plants. There were a number of tetraploid plants, however, which were as early as the diploid plants. For instance,

TABLE IV

Redwing tetraploid and diploid plants grown in greenhouse
(seeded August 4, 1941)

Plant No.	Chromosome No.	Days to 1st flower	(mm.) Flower diam.	(mm.) Petal		Pollen diam. mu*	Stomatal size*		Stomatal area	Height at maturity, inches	No. of bolls	(mm.) Mean boll diam.	Mean No. seeds per boll
				w	l		w	l	w x l				
47-1	--	177	19	8.0	12.0	67.3	4.4	9.0	39.60	30	21	7.6	2.9
150-1	57	182	20	7.0	11.0	71.6	4.2	9.2	38.64	--	11	7.4	1.3
64-3	60	175	--	-	--		4.6	8.8	40.48	--	--	-	-
28-3	60	143	18	8.0	11.0	68.8	4.8	8.8	42.24	27	37	7.4	2.6
65-1	60	151	22	8.5	12.0	68.1	4.1	7.3	29.93	27	47	7.9	2.0
64-1	60	145	19	7.5	11.0	67.7	4.2	8.7	36.54	30	46	7.6	2.2
64-2	60	160	23	9.0	13.5	71.6	4.8	9.1	43.68	--	19	7.2	1.2
65-2	60	174	22	8.0	12.0	68.4	4.8	9.2	44.16	--	26	7.2	1.4
81-2	--	141	18	7.0	11.0	67.0	5.1	9.2	46.92	--	--	-	-
81-1	60±	133	18	7.5	11.0	67.9	4.7	9.2	43.24	28	29	7.6	2.6
28-4	60±	174	20	8.5	13.5	68.4	3.7	7.0	25.90	20	23	7.5	1.9
29-1	60	175	24	8.5	13.0	69.0	4.8	9.7	46.56	27	45	7.5	2.0
28-1	60	164	21	8.0	12.0	67.3	4.4	9.7	42.68	25	50	7.6	2.0
28-2	59±	189	20	8.0	13.0	69.4	4.4	9.8	43.12	24	10	7.2	2.0
Mean		163.0	20.3	8.0	12.0	68.6	4.5	8.9	40.26	26.4	30.3	7.5	2.0

Mean Volume of pollen grains 169,000.0 cu.mu

TABLE IV (Continued)

Plant No.	Chromosome No.	Days to 1st flower	(mm.) Flower diam.	(mm.) Petal		Pollen diam. mu*	Stomatal size*		Stomatal area	Height at maturity, inches	No. of bolls	(mm.) Mean boll diam.	Mean No. seeds per boll
				w	l		w	l	w x l				
75-1	30	130	13	7.0	9.0	53.4	4.6	7.6	34.96	30	23	6.4	6.3
Ck-1	30	141	13	5.5	9.5	56.2	3.5	6.4	22.40	27	32	6.5	7.6
75-4	30	146	13	7.0	9.0	56.9	3.7	6.6	24.42	28	57	6.1	7.1
Ck-4	30	141	15	7.0	9.0	54.9	3.4	7.1	24.14	--	38	6.6	7.3
69-4	30	142	13	6.0	10.0	55.1	3.2	7.0	22.40	29	46	6.5	7.4
Ck-2	30	150	13	6.0	9.0	54.4	3.7	6.6	24.42	28	34	6.1	6.3
Ck-3	30	132	13	7.0	9.0	54.3	4.0	6.8	27.20	--	44	6.5	6.9
69-3	30	175	14	7.0	10.0	54.6	4.1	7.8	31.98	--	35	6.8	9.5
69-1	30	147	14	7.0	10.0	56.1	3.8	6.4	24.32	--	45	6.7	7.4
75-2	30	134	15	7.0	10.0	55.3	3.4	6.2	21.08	23	7	6.4	3.1
75-3	--	148	15	7.0	10.0	55.2	3.8	6.7	25.46	27	52	6.4	7.8
69-2	30	146	14	6.0	9.0	57.7	3.6	6.5	23.40	--	41	6.7	8.3
75-5	30	150	14	8.0	10.0	54.7	3.8	7.4	28.12	--	34	6.5	7.8
Mean		144.7	13.7	6.7	9.5	55.3	3.7	6.8	25.75	27.4	37.5	6.5	7.1
Diff. (tetraploid - diploid)		18.3	6.6	1.3	2.5	13.3	0.8	2.1	14.51	-1.0	-7.2	1.0	-5.1
% inc. tetraploid over dipl.		12.6	52.0	19.4	26.3	24.0	21.6	30.9	56.3	-3.6	-19.2	15.4	-71.8

Mean volume of diploid pollen grains 88,000 cu.mu
Vol. difference (tetraploid - diploid) 80,900 cu.mu
% increase in volume of tetraploid over diploid 91.9

* Each entry represents the mean of 10 measurements.
For stomatal size 1 unit = 3.7 mu.

tetraploid plant 81-1 was over nine days earlier than the diploid mean and was only three days later than the earliest diploid.

Flower diameter increased 52.0%, petal width 19.4% and petal length 26.3% in the transition from the diploid to the tetraploid condition.

Pollen size was remarkably constant in both the diploid and the tetraploid plants. The mean diameter of the tetraploid pollen was 27.2% greater than the diploid pollen. If the pollen grains were perfect spheres, the mean volume of the tetraploid grains would be 90.9% greater than that of the diploid grains. This would approach the doubled volume that might be expected from the doubled chromosome number.

Stomatal width in tetraploid plants increased 21.6%, length 30.9%, and the area computed by multiplying the length by the width increased 56.3%.

Height at maturity measured on the plants available showed the diploids 3.6% taller than the tetraploids.

The mean number of bolls borne by the tetraploids was 19.2% less than that of the diploids, but the mean diameter of the tetraploid bolls was 15.4% greater than that of the diploids. The diploid bolls contained a mean of 7.1 seeds per boll, in contrast to a mean of 2.0 seeds in the tetraploid bolls--a reduction in fertility of 71.8%. Some of the tetraploid plants were slightly more fertile than others but this may be due only to random variation. Light, humidity, and temperature in the greenhouse varied greatly while these plants

were setting seed and may have had a pronounced effect on their seed setting ability.

A test similar to the one just described was sown on December 17, 1941 (Table V). Since there were only three diploid plants included, the mean values for the diploids are not so reliable as in the previous test, but growing conditions were better.

Among the tetraploid plants there were variations in the number of days from seeding to first flower. The progeny of the original shoot 81 seemed to be earlier than the others in both this and the previous test. The mean of days from seeding to first flower is 1.3% greater for the diploid than for the tetraploid plants. This disagreement with the former results may be explained by the small number of diploid plants used for comparison and by the better growing conditions of the second test. Difficult growing conditions may act more adversely on the tetraploid plants than on the diploid plants. In a field test of tetraploids and diploids no difference between plants could be seen. The whole test flowered at the same time--58 days after seeding.

The flower diameter of the tetraploid was 31.6% greater than that of the diploid and the flowers of the diploid were proportionately larger in the second test in relation to the flowers of the tetraploid than in the first test. Petal size was 22.8% wider and 23.0% longer on the tetraploid than on the diploid plant. This contrast in flower size is illustrated in Figure 4.

TABLE V

Redwing tetraploid and diploid plants grown in greenhouse
(seeded Dec. 17, 1941)

Plant No.	Chromosome No.	Days to 1st flower	Flower diam. mm.	Petal (mm.)		Pollen diam. mu*	Stomatal size*		Stomatal area w x l	Height Jan.17 mm.	Height at maturity, inches	No. of bolls	Mean boll diam. mm.	Mean No. seeds per boll
				w	l		w	l						
81-3	--	93	24	8.5	14.0	67.1	4.3	8.2	35.26	93	19.0	53	7.4	1.9
81-4	60	96	24	9.0	14.0	72.5	4.1	8.5	34.85	26	22.0	27	7.0	1.6
81-6	60	100	24	8.0	15.0	70.1	4.2	8.3	34.86	45	20.5	48	7.3	2.0
81-7	60	95	25	9.0	15.0	69.0	4.4	8.2	36.08	75	25.0	31	7.7	2.0
150-2	59	103	23	8.0	14.0	68.7	4.2	8.4	35.28	53	24.0	33	7.1	1.8
150-3	--	112	24	9.0	14.0	71.4	-	-	---	18	20.5	15	7.3	1.8
150-4	64	111	24	8.0	15.0	71.9	-	-	---	20	23.0	17	7.5	2.4
150-5	60±	100	23	8.0	15.0	69.5	4.1	8.6	35.26	97	23.0	40	7.7	2.8
150-6	60	98	24	9.0	14.0	70.5	4.4	8.2	36.08	88	28.0	40	7.6	2.8
28-5	60	98	24	9.0	14.0	70.9	4.2	8.4	35.28	83	26.0	11	7.2	2.1
61(a)-1	60	100	23	9.0	15.0	69.1	4.2	8.2	34.44	85	20.0	28	7.5	1.1
56-1	--	104	24	9.0	15.0	71.0	-	-	---	30	26.0	15	7.3	2.9
56-3	60	101	22	9.0	14.0	71.0	4.5	8.4	37.80	74	22.0	53	7.1	2.3
59-1	62	101	23	9.0	14.0	67.0	4.2	8.6	36.12	77	19.0	36	7.3	2.6
59-2	60	101	23	8.0	14.0	68.3	4.1	8.6	35.26	42	30.0	38	7.5	2.4
59-3	--	100	24	9.0	15.0	72.4	4.4	8.4	36.96	68	26.0	56	7.5	2.9
15-4	58	95	24	8.0	14.0	68.3	4.4	8.4	36.96	100	27.0	32	7.3	2.2
59-5	64	103	24	9.0	15.0	69.8	4.1	8.1	33.21	20	27.0	37	7.4	2.2
Mean		101.0	23.7	8.6	14.4	69.9	4.3	8.4	35.6	60.8	23.7	33.9	7.4	2.2

TABLE V (Continued)

Plant No.	Chromosome No.	Days to 1st flower	Flower diam. mm.	Petal(mm.) w	Petal(mm.) l	Pollen diam. mu*	Stomatal size*		Stomatal area w x l	Height Jan.17 mm.	Height at maturity, inches	No. of bolls	Mean boll diam. mm.	Mean No. seeds per boll
Ck-6	30	104	18	7.0	12.0	56.6	3.0	6.0	18.0	40	26.0	26	6.5	8.4
Ck-7	30	101	18	6.0	12.0	55.2	3.0	6.4	19.2	45	31.0	76	6.4	7.3
Ck-9	30	102	18	8.0	11.0	59.1	3.0	6.2	18.6	30	27.0	28	6.7	8.6
Mean		102.3	18.0	7.0	11.7	56.9	3.0	6.2	18.6	38.3	28.0	43.3	6.5	8.1
Diff. (tetraploid - diploid)		-1.3	5.7	1.6	2.7	13.0	1.3	2.2	17.0	22.5	-4.3	-9.4	0.9	-5.9
% inc. tetraploid over dipl.		-1.3	31.6	22.8	23.0	22.8	43.3	35.5	91.4	58.7	-15.4	-21.7	13.8	-72.8

Mean volume of tetraploid pollen grains	178,000.0 cu.mu
Mean volume of diploid pollen grains	95,900.0 " "
Vol. difference (tetraploid - diploid)	82,100.0 " "
% increase in volume of tetraploid over diploid	85.6

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* Each entry represents the mean of 10 measurements. For stomatal size 1 unit = 3.7 mu.

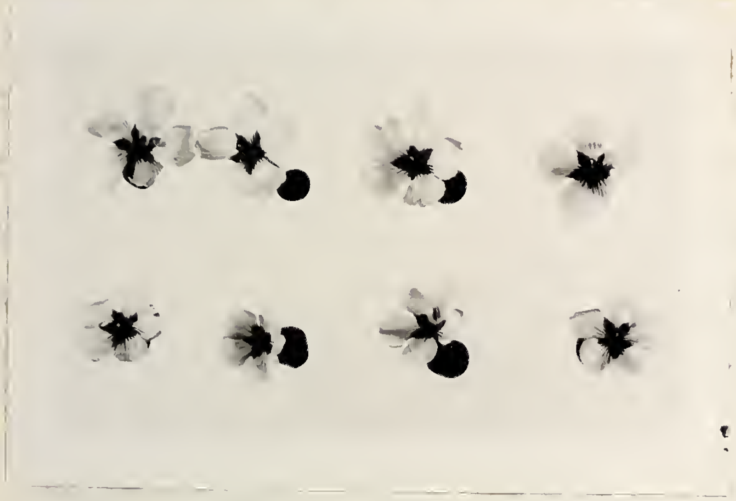

Figure 4

Illustration of the difference in size of flowers from a tetraploid Redwing plant (above) and a diploid Redwing plant (below).

Pollen diameter was 22.9% greater for the tetraploid than for the diploid plant. The volume computed on the same basis as for the first test was 86.4% greater for the tetraploid than the diploid pollen grains. The difference in diameter is illustrated by a comparison of Figure 5 and Figure 6.

Stomatal width was 43.3% and the length 35.5% greater on the tetraploid than on the diploid plants. The calculated stomatal area derived by multiplying length by width was 91.4% greater for the tetraploid than for the diploid. The small number of diploid plants examined as well as variation in size of stomata may be responsible for the difference between this result and that obtained in the test seeded August 4th. Contrast in size between stomata of a diploid and a tetraploid is shown in Figures 7 and 8.

The rate of growth was checked by measurements made 31 days after seeding and it was found that the tetraploid plants averaged 58.7% taller than the diploids. Because of the small number of diploids involved, this means very little, but it does indicate that the tetraploid plants are as rapid in growth as the diploid plants. The comparative heights at maturity differed from those one month after seeding in that the mature tetraploid plants were 15.4% shorter than the diploid plants, which may indicate a tendency in the tetraploid plants to be shorter.

The mean number of bolls per plant was 21.7% less on the tetraploid plant than on the diploid, but the mean diameter of the tetraploid bolls was 13.8% greater. The mean fertility

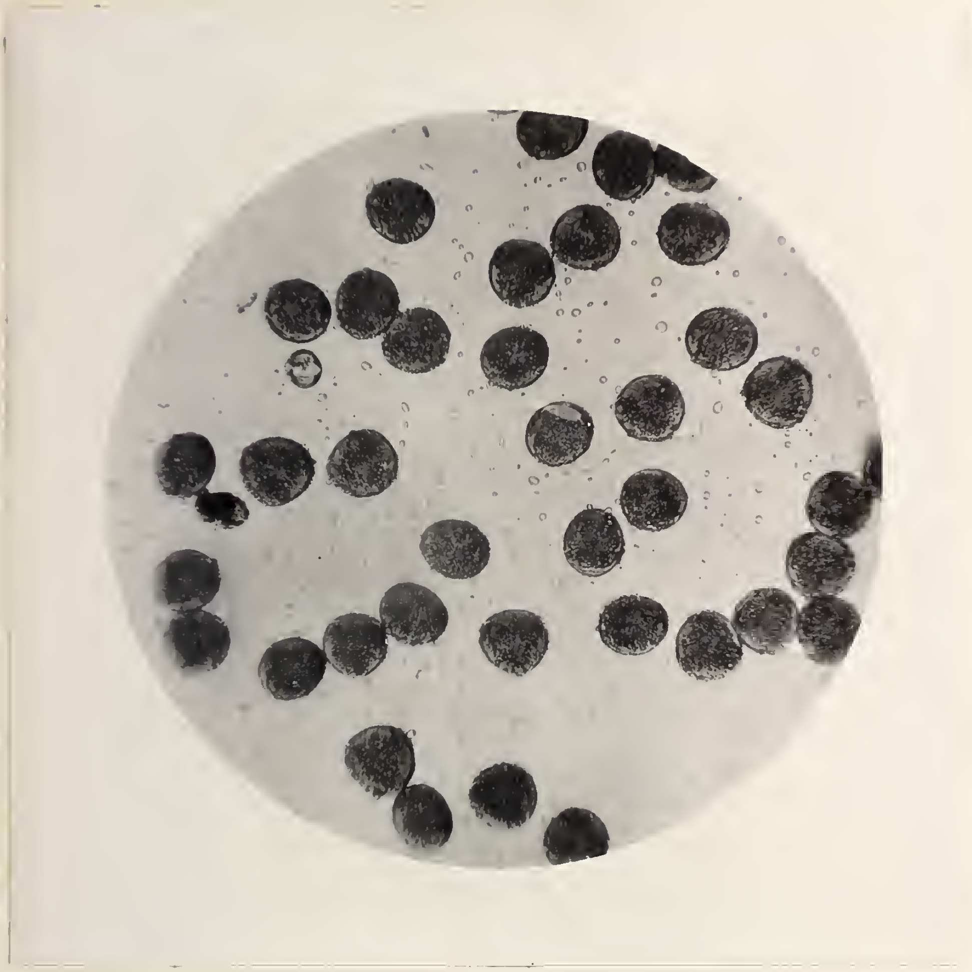

Figure 5

Microphotograph of pollen grains of
diploid Redwing. x 150

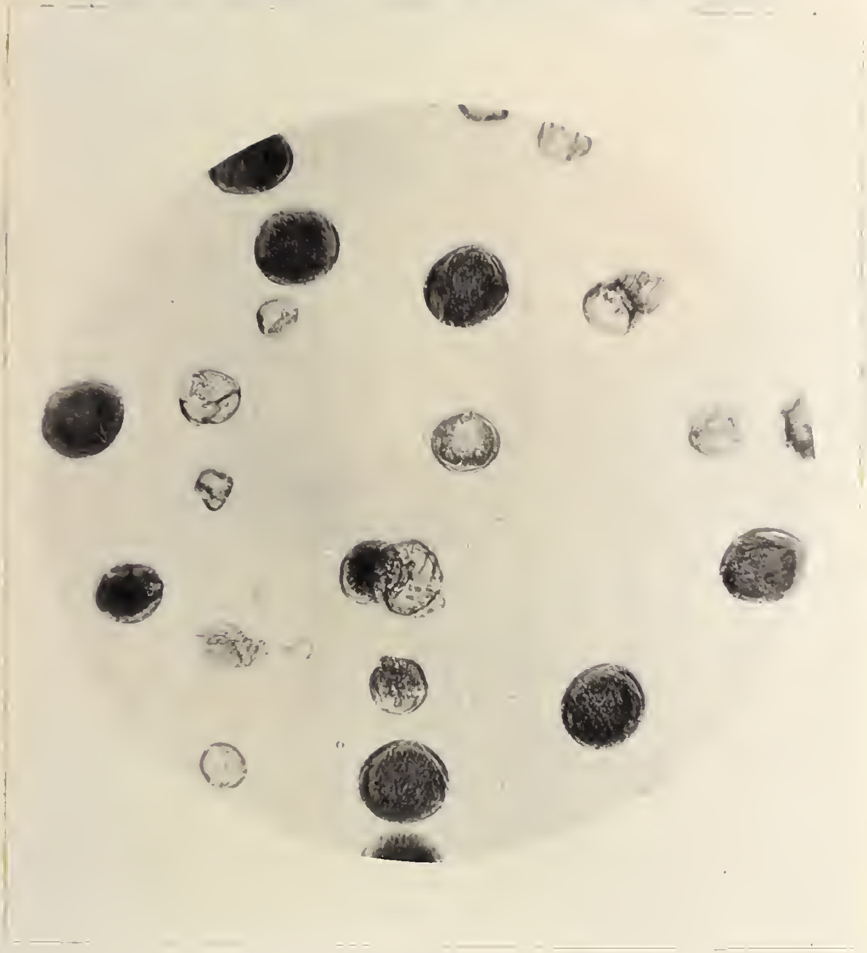

Figure 6

Microphotograph of pollen grains of
tetraploid Redwing. x 150

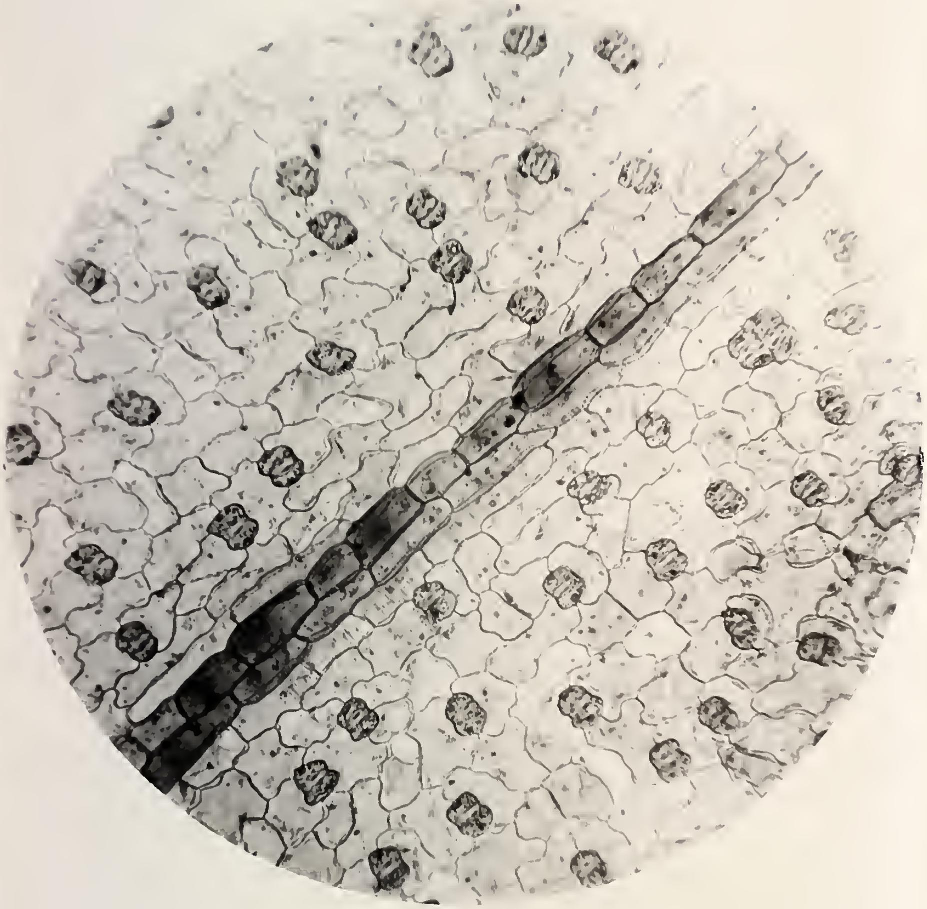

Figure 7

Microphotograph of lower epidermis stripped from a leaf, illustrating the size of stomata found in diploid Redwing plants. x 150

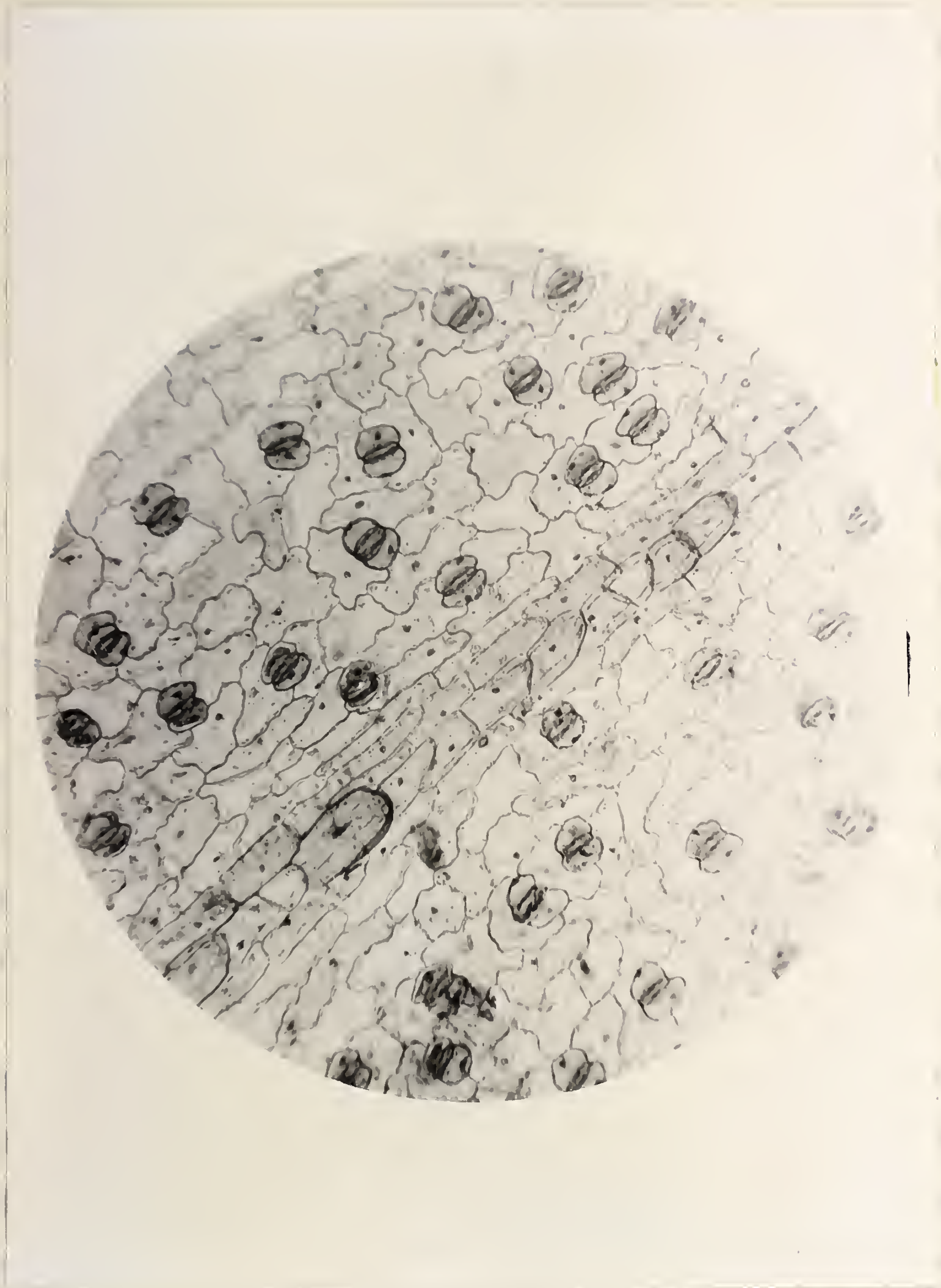

Figure 8

Microphotograph of lower epidermis stripped from a leaf, illustrating the large stomata found in tetraploid Redwing plants. x 150

of the tetraploid bolls was reduced 72.8% below the mean for the diploid bolls. Again the variation between mean fertility for different tetraploid plants was small, but there were some bolls containing five or six seeds and therefore there may be some possibility of selecting more fertile plants by propagating the seed from these bolls.

The increased weight of the tetraploid over the diploid seed was determined by weighing 500 seeds from the diploid and 500 seeds from the tetraploid plants harvested during the summer of 1942, these being the plants indicated in Tables IV and V. Converted to 1000-kernel weight the diploid seed weighed 4.776 gm. and the tetraploid 5.644 gm., representing an increase in weight of 18.1%, or approximately the difference in 1000-kernel weight between Redwing and Bison as represented in Table I. The differences in size of boll and seed are shown in Figure 9.

Observations of enlarged stomata and pollen grains have been used quite commonly to distinguish between tetraploid and diploid plants. In this study, as previously mentioned, stomatal size was found to differ between leaves and between locations on a leaf. One instance of small stomata on a plant with 60± chromosomes is noted in plant Number 28-4, Table IV. It is evident that this method of distinguishing tetraploid plants would not be infallible.

From observations on the plant characters in Tables V and VI, the diameter of the pollen grain appears to be the most constantly enlarged part of the tetraploid plant.

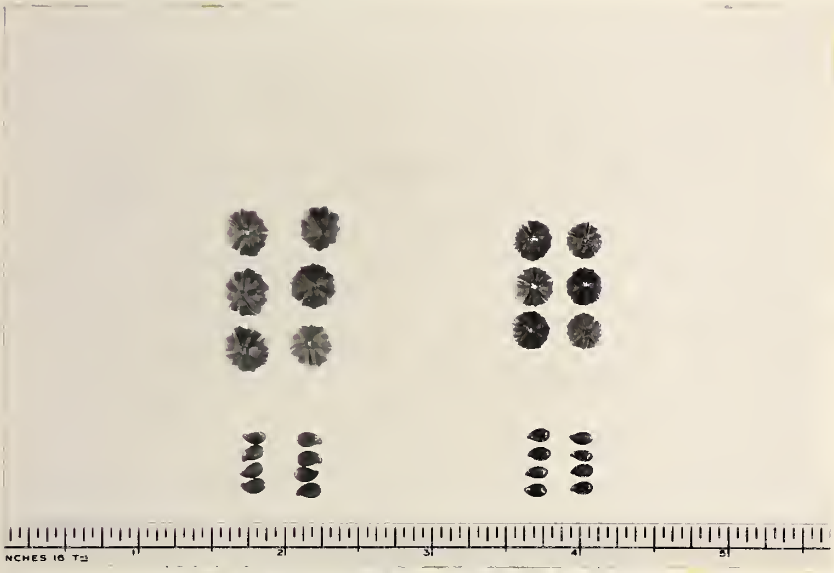

Figure 9

Bolls and seed of Redwing flax, illustrating the difference in size between the tetraploid form on the left and the diploid form on the right.

In Table VI the range of pollen diameter is shown to be approximately twice as large in the tetraploid as in the diploid. The mean diameter in each of the two proven tetraploids is remarkably constant. Examination of Figures 5 and 6 supports the observations made in Table VI except that in the tetraploid pollen more abortion, with consequent lessened percentage viability, seems to be present.

The small variations in mean pollen diameter among different varieties and crosses of diploid types and also among the corresponding tetraploid types are shown in Table VII.

From these observations it may be concluded that measurements of the diameters of pollen grains give a reliable indication of the tetraploid or diploid character of the plant.

Chromosome Numbers of Parent Varieties and Tetraploid F_2 Hybrids

Pollen mother cells of each of the parents involved in the crosses were examined, and in every case the haploid number was established as 15. Illustrations are given of a pollen mother cell of each variety at a stage which allows the haploid chromosome number to be distinguished (Figures 10, 11, 13, and 15). A number of pollen mother cells of tetraploid Redwing were examined. A polar view (Figure 10) shows 30 bivalents, but the presence of univalents, trivalents, or

TABLE VI

Viability and diameter of pollen grains of tetraploid and diploid Redwing

Plant No.	Chromosome number	Number examined	% viable	Range in diam. of viable pollen mu	Mean diam. of viable pollen mu
64-1	60	100	88	53 - 72	67.6
28-1	60	100	82	52 - 75	68.6
47-1	--	100	92	58 - 76	68.9
Check-2	30	100	98	48 - 58	54.2

TABLE VII

Comparison of diameter of diploid and tetraploid pollen grains

	Diameter of diploid pollen grains*					Diameter of tetraploid pollen grains*				
					Mean					Mean
L-33-1	57.2	57.8	57.7	59.2	58.0					
L-35-23	59.0	58.3	56.5	59.7	58.4					
Bison	57.4	55.2	56.0	56.5	56.3					
Redwing	56.4	55.4	55.1	54.2	55.3	69.2	69.0	70.9	69.4	69.6
L-33-1 x Redwing	54.9	56.0	56.9	58.1	56.4	69.7	70.2	71.1	70.5	70.4
L-35-23 x Redwing	55.8	54.1	55.8	55.4	55.3	69.5	68.1	69.1	69.2	69.0
Bison x Redwing	55.4	54.2	57.2	53.3	55.0	68.2	69.2	68.8	68.8	68.8

* Each entry is from a particular plant and represents the mean of 10 readings.

1 unit of measurement = 1 mu

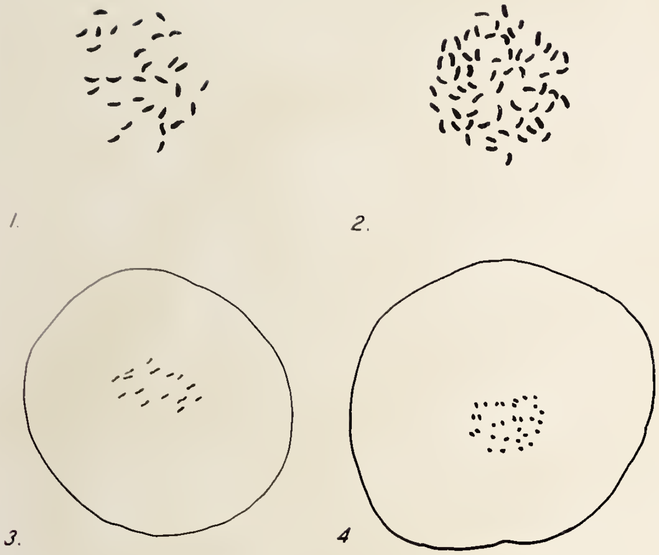

Figure 10

1. Somatic metaphase plate of diploid form of Redwing (2n = 30) x 2400

2. Somatic metaphase plate of tetraploid form of Redwing (4n = 60). x 2400

3. Pollen mother cell of diploid form of Redwing at metaphase of first division of meiosis showing 15 bivalents. x 1000

4. Pollen mother cell of tetraploid form of Redwing at metaphase of first division of meiosis showing 30 bivalents. x 1000

The above drawings were made with the aid of a camera lucida

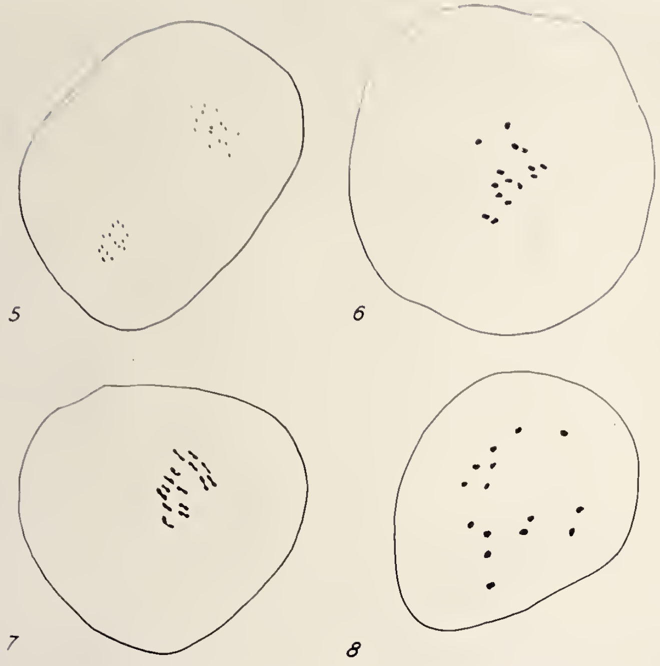

Figure 11

5. Pollen mother cell of the diploid form of Crown at the metaphase of second division of meiosis (n = 15)

6. Pollen mother cell of the diploid form of Indian Type 35 at the metaphase of first division of meiosis (n = 15)

7. Pollen mother cell of the diploid form of L-33-14 at the metaphase of first division of meiosis (n = 15)

8. Pollen mother cell of the diploid form of Royal at the early metaphase of first division of meiosis (n = 15)

The above were drawn with the aid of a camera lucida. x 1000

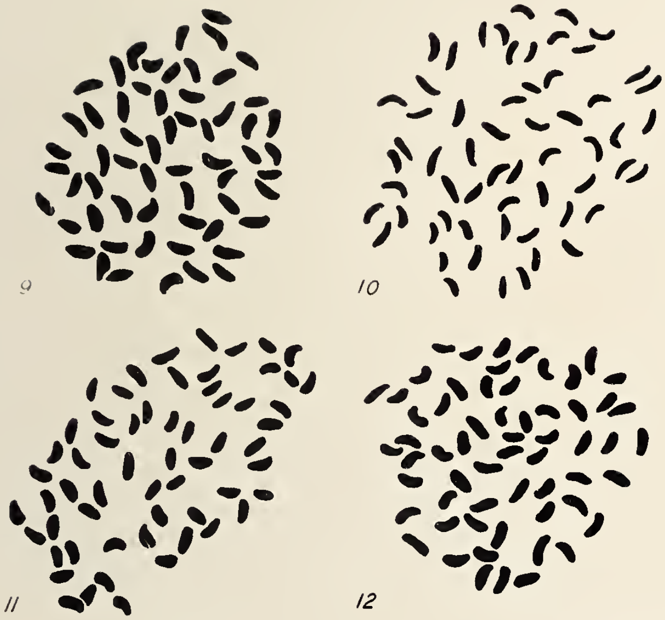

Figure 12

9. Somatic metaphase plate of tetraploid form of the F_2 hybrid Crown x Redwing (4n = 60)

10. Somatic metaphase plate of tetraploid form of the F_2 hybrid Indian Type 35 x Redwing (4n = 60)

11. Somatic metaphase plate of tetraploid form of the F_2 hybrid L-33-14 x Redwing (4n = 60)

12. Somatic metaphase plate of tetraploid form of the F_2 hybrid Royal x Redwing (4n = 60)

The above drawings were made with the aid of a camera lucida. x 2400

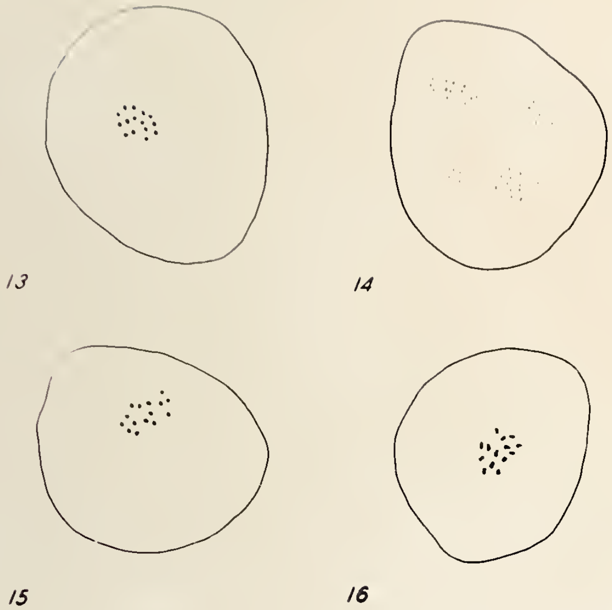

Figure 13

13. Pollen mother cell of diploid form of L-33-1 at metaphase of the first division of meiosis showing 15 bivalents

14. Pollen mother cell of diploid form of Bison at late anaphase of the second division of meiosis showing the haploid number of 15

15. Pollen mother cell of diploid form of L-35-23 at metaphase of the first division of meiosis showing 15 bivalents

16. Pollen mother cell of diploid form of Pale Blue at metaphase of the first division of meiosis showing 15 bivalents

The above drawings were made with the aid of a camera lucida. x 1000

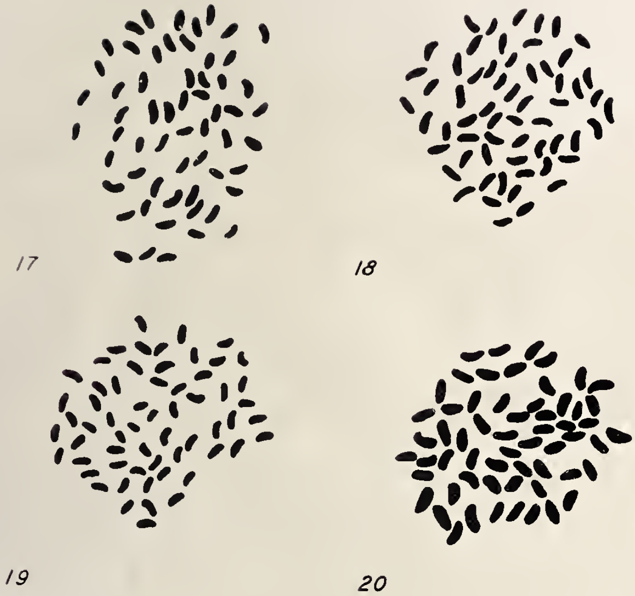

Figure 14

17. Somatic metaphase plate of tetraploid form of the F_2 hybrid L-33-1 x Redwing (4n = 59)

18. Somatic metaphase plate of tetraploid form of the F_2 hybrid Bison x Redwing (4n = 60)

19. Somatic metaphase plate of tetraploid form of the F_2 hybrid L-35-23 x Redwing (4n = 60)

20. Somatic metaphase plate of tetraploid form of the F_2 hybrid Plae Blue x Redwing (4n = 60)

The above drawings were made with the aid of a camera lucida. x 2400

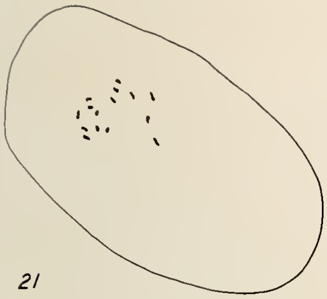

Figure 15

21. Pollen mother cell of diploid form of Hoshangabad at metaphase of the first division of meiosis showing 15 bivalents

The above drawing was made with the aid of a camera lucida. x 1000

tetravalents was not detected. The unequal distribution of chromosomes at first or second division of meiosis, however, took place in some instances as is evidenced by the occurrence in some cases of five microspores with five-celled conditions instead of the normal tetrad. The large range of diameter previously mentioned among tetraploid pollen grains may also be due to this unequal distribution of chromosomes.

Counts of somatic chromosomes were made on the tetraploid F_2 hybrids (Table VIII) and the chromosome numbers found to deviate very little from the expected number of 60. Somatic metaphase plates of chromosomes from one plant of each of the crosses are shown in Figures 12 and 14.

A Preliminary Comparison of Fertility and Size of Seed and Boll of Tetraploid and Diploid Shoots of Treated Plants of F_1 Hybrids and of Redwing

Among treated plants the tetraploid shoots were separated from the diploid shoots by examination of the pollen grains from the flowers (Table IX). The severity of treatment might have had some effect on the morphological characters of the treated plant but, since both the tetraploid and diploid shoots set seed under the same conditions of light, humidity, and temperature, their fertility may be compared.

The decrease in fertility ranged from the highest (75.6%) in Crown x Redwing and 72.0% in the variety Redwing to 42.1% in Indian Type x Redwing and 43.9% in Pale Blue x

TABLE VIII

Chromosome counts on tetraploid F_2 hybrids

Cross	Chromosome numbers of different plants								
L-35-23 x Redwing	60±	60	60±	60	60	60	60	60	59
Bison x Redwing	60±	60	60	60	59	60±	62	60	61
L-33-1 x Redwing	61	60±	59	60	59	60	60		
Royal x Redwing	60	60	60						
Indian Type 35 x Redwing	60	60	60						
Hoshangabad x Redwing	60	60							
L-33-14 x Redwing	60	59							
Crown x Redwing	60	60							
Pale Blue x Redwing	60	60							

TABLE IX

Fertility and boll comparisons for diploid and tetraploid shoots from treated seedlings of Redwing variety and of F_1 hybrids

F_1 hybrid or variety	No. of bolls on shoot	Av. No. seeds per boll	Av. diam. of bolls mm.
(2n) Redwing	6	7.5	7.2
(4n) "	7	2.1	7.2
% decrease in fertility of (4n) under (2n)		72.0	+0.0*
(2n) Pale Blue x Redwing	13	8.2	6.6
(4n) " " x "	3	4.6	7.7
% decrease in fertility of (4n) under (2n)		43.9	+16.6
(2n) Indian Type 35 x Redwing	12	5.7	6.9
(4n) " " " x "	10	3.3	7.6
% decrease in fertility of (4n) under (2n)		42.1	+10.1
(2n) L-35-23 x Redwing	21	5.8	7.3
(4n) " x "	23	2.8	8.9
% decrease in fertility of (4n) under (2n)		51.7	+21.9
(2n) Royal x Redwing	15	8.2	6.6
(4n) " x "	9	2.8	7.7
% decrease in fertility of (4n) under (2n)		-65.8	+16.6
(2n) Bison x Redwing	4	7.5	6.0
(4n) " x "	21	3.0	7.4
% decrease in fertility of (4n) under (2n)		-60.0	+23.3
(2n) L-33-1 x Redwing	21	7.6	7.0
(4n) " x "	11	3.4	8.4
% decrease in fertility of (4n) under (2n)		-55.2	+20.0
(2n) Crown x Redwing	9	7.4	6.5
(4n) " x "	19	1.8	6.6
% decrease in fertility of (4n) under (2n)		-75.6	+1.5
(2n) L-33-14 x Redwing	6	8.2	6.6
(4n) " x "	8	2.3	7.3
% decrease in fertility of (4n) under (2n)		-71.9	+10.6
(2n) Hoshangabad x Redwing	6	4.7	6.9
(4n) " x "	1	2.0	8.0
% decrease in fertility of (4n) under (2n)		-57.4	+15.9

(2n) = diploid

(4n) = tetraploid

* The numbers preceded by the plus sign represent the increases in boll diameter of the tetraploid over the diploid.

Redwing. Very little can be concluded definitely from this evidence but the possibility of increased fertility of the hybrids over the pure variety is indicated.

The relative sizes of tetraploid and diploid seed of all crosses are illustrated at natural size (Figures 16, 17, 18, and 19). In most cases the increase is quite marked.

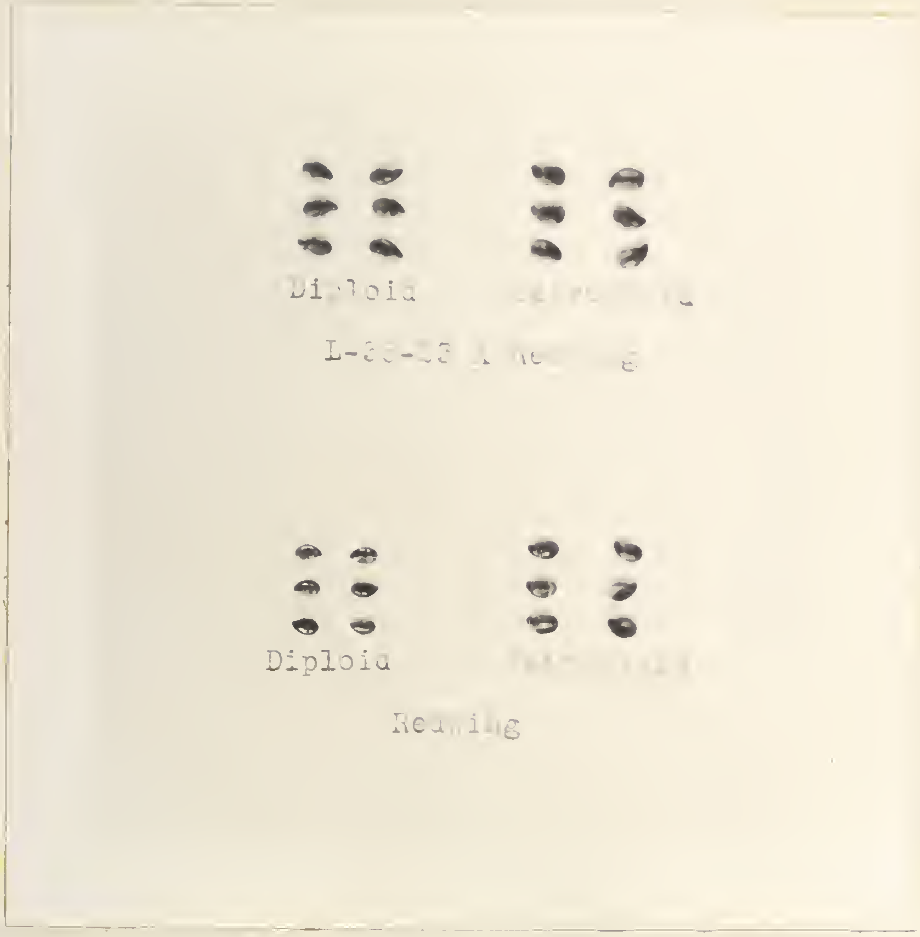

Figure 16

Illustration of contrast in size between
diploid and tetraploid seeds
(natural size)

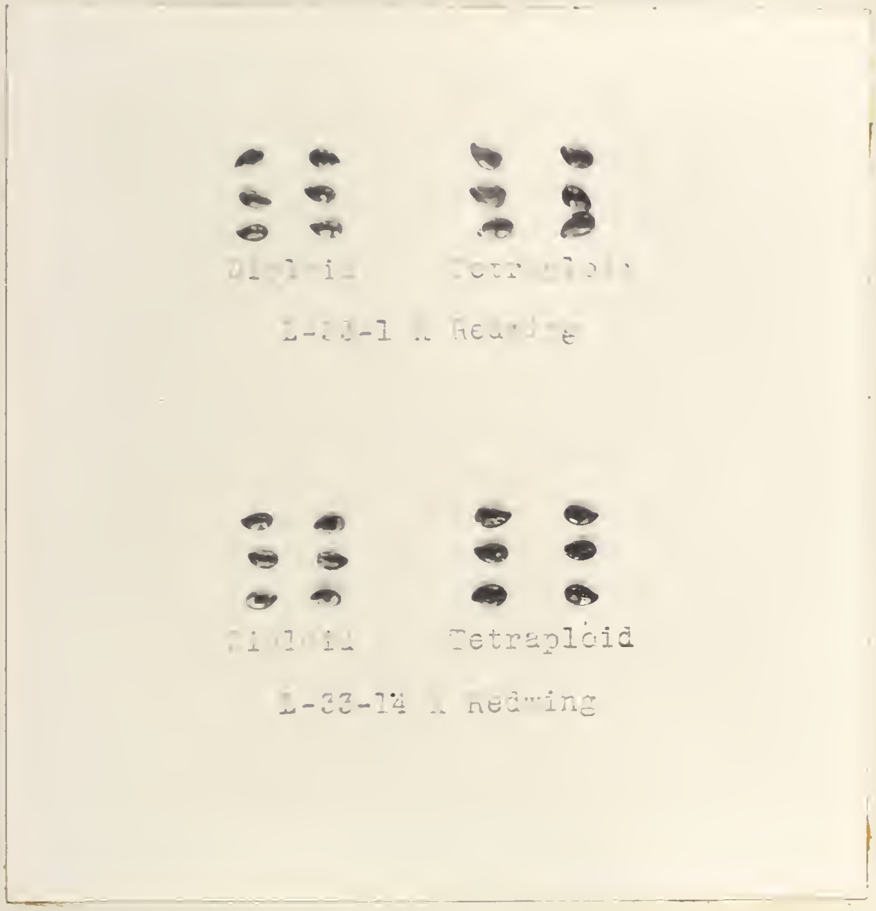

Figure 17

Illustration of contrast in size between
diploid and tetraploid seeds
(natural size)

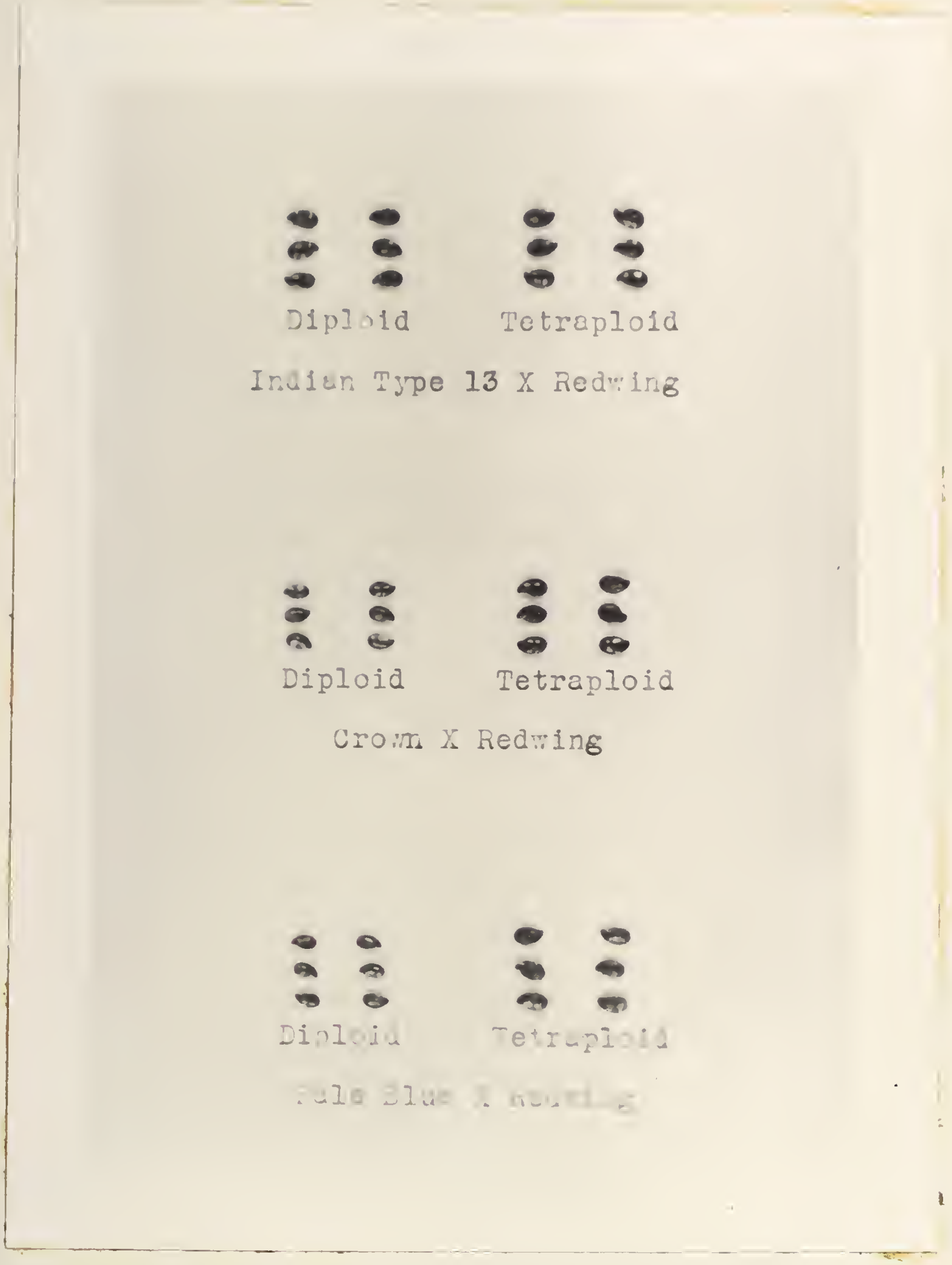

Figure 18

Illustration of contrast in size between
diploid and tetraploid seeds
(natural size)

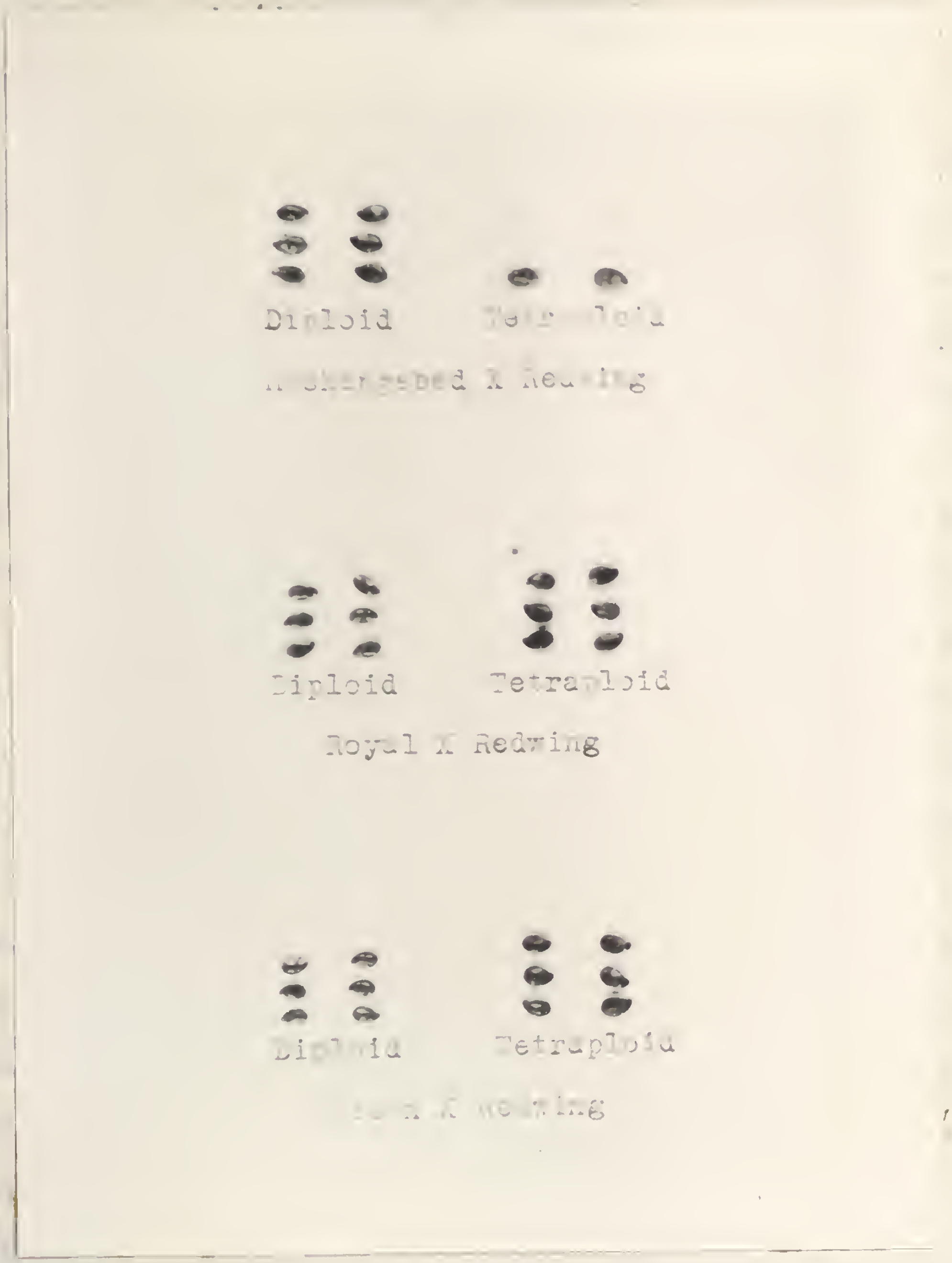

Figure 19

Illustration of contrast in size between diploid and tetraploid seeds (natural size)

DISCUSSION OF RESULTS

From this study it may be concluded that the most satisfactory method used to produce tetraploid flax shoots is the application of colchicine in lanolin emulsion to the epicotyl of the seedling as it emerges from between the cotyledons. Lutkov (10) obtained higher percentages of tetraploids by means of a modified germinating seed treatment, while Simonet and Chopinet (18), using a germinating seed treatment, secured poorer results than those indicated in this investigation.

The flax varieties used in this study were found to have the constant somatic chromosome number of 30, or the haploid number of 15 which agrees with the findings of Tammes (21), Vilmorin and Chopinet (22), Kikuchi (8), and Dillman (4). The diploid somatic numbers were found to be quite stable and did not differ among plants of any variety studied as did those reported by Muntzing and Runquist (13). The tetraploid somatic chromosome numbers differed from those reported by Lutkov (10) but represented, as did his, the tetraploid condition of the chromosomes of the original diploid.

In examinations of the pollen mother cells of the tetraploid plant, 30 bivalents were observed, which agreed with Simonet's, Chopinet's and Souilijaert's (19) findings. The displacing of the tetrads with more than four cells was also noted by these investigators. These irregularities at meiosis led to a sterility of the tetraploid plants similar to

that reported by Lutkov (10) and Ray (16). There is some indication in this study that the fertility of the heterozygous tetraploid may be higher than that of the homozygous doubled plant. This would be expected from the work of Randolph (15) and Sparrow, Ruttle and Nebel (20). Up to the present time, it has not been possible to compare the fertility of these tetraploid plants in the greenhouse with those in the field, but there is a probability of increased fertility in the field similar to results obtained by Newcomer (14) working with Cosmos.

The enlarged parts characteristic of tetraploid plants were evident in the tetraploid flax plants. Examination of pollen grain diameter was found to give a reliable criterion on which to decide the tetraploid or diploid character of the plant. This was true in every case, in contradistinction to the findings of Lutkov (10). L. usitatissimum L. responded to doubling of its chromosome complement without delaying maturity or dwarfing as sometimes occurs when the chromosome number of a species is doubled, although the fertility of the plant was greatly reduced. The larger seed produced from the tetraploid plants fulfils the purpose for which this study was undertaken, but the lack of fertility exhibited necessitates the continued selection of fertile types as practised by Kostoff (9) if any practical application of this finding is to be made.

SUMMARY

1. A review of the literature reveals that a number of workers have produced tetraploid forms of Linum usitatissimum L. which, although large-seeded, were lacking in fertility. Several investigators achieved increased fertility in tetraploid forms of other plant species by increasing their heterozygosity.

2. Among the treatments used, application of colchicine in lanolin emulsion to the epicotyl of the seedling was the most efficacious in the production of tetraploids.

3. Tetraploid forms of Redwing and of the F_1 hybrids of nine crosses involving that variety showed the usual tetraploid characteristics of large stomata, large flowers, thickened leaves, large pollen grains, and no decrease in vigor, but unfortunately greatly lessened fertility, particularly in the case of Redwing.

4. Measurement of the pollen grains was found to be a reliable method of separating the tetraploid from the diploid plants, the tetraploid grains being almost double the volume of the diploid grains. The large pollen measurements corresponded to the somatic chromosome numbers of the tetraploid plants which were in most cases 60 but in some 1, 2, or 3 more or less; while the small measurements indicated that the plant in question was a diploid with chromosome number 30.

5. The haploid numbers of the ten varieties (the parents of the above-mentioned nine crosses) were determined by examination of the pollen mother cells and found to be 15 in every variety. In examinations of pollen mother cells of tetraploid Redwing, a count of 30 bivalents was made and irregularities in meiosis observed. Somatic counts made on the F_2 tetraploid hybrids of eight crosses deviated very little from the expected 60.

6. Comparison of tetraploid and diploid shoots from treated seedlings indicated the possibility of increased fertility of the tetraploid hybrid over the homozygous tetraploid Redwing.

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